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TITLE: A Novel Field-Deployable Point-of-Care Diagnostic Test for Cutaneous Leishmaniasis

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RECIPIENT: The Henry M. Jackson for the Advancement of Military Medicine
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14. ABSTRACT

Background. Leishmaniasis is caused by the protozoan *Leishmania* and is generally transmitted by the bite of sand flies of the genus *Lutzomyia* or *Phlebotomus*. The disease has significant global impact, producing 10-20 million cases of leishmaniasis worldwide. Cutaneous leishmaniasis (CL) is characterized by chronic skin ulcers that can impact the individual's functional status, lead to expensive and untimely treatment, and result in disfiguring scarring. Leishmaniasis causes a spectrum of diseases that include localized cutaneous leishmaniasis (LCL), and destructive nasal and oropharyngeal lesions of mucosal leishmaniasis (ML). LCL in the New World is most commonly caused by species of the *Viannia* subgenus (*L. braziliensis*, *L. panamensis*, *L. guyanensis*, *L. peruviana*) and to a lesser extent by species of the *Leishmania* subgenus (*L. mexicana*, *L. amazonensis*). Historically, the leishmaniasis have had significant impact on military operations. Thousands of cases of visceral and cutaneous leishmaniasis occurred in soldiers in World Wars I and II. Military training and combat operations resulted in cases of CL in soldiers (USA, UK) deployed to Central America. More recently (2003-2004), CL was reported in almost 1,200 members of the U.S. Armed Forces deployed to Iraq and Afghanistan, and the infection is an ongoing concern in the OEF/OIF veteran population. Unpublished information indicates that the number of military personnel with cutaneous leishmaniasis could exceed 3,000.

Rationale. A major challenge in the diagnosis of leishmaniasis is that the disease occurs in remote and resource-limited areas of the world with poor or nonexistent primary health infrastructure. This also could be true during military field operations and training exercises where sophisticated laboratory equipment and medical personnel are scarce or not available. For CL or ML, scrapings of dermal tissues or punch biopsies of the lesions are necessary and the diagnostic sensitivity by histopathology, microscopy of smears or culture could be unacceptably low (40-70%). The highly sensitive PCR method cannot be implemented in resource-poor settings due to the high costs, personnel training and need of sophisticated equipment. Therefore, novel methods to detect leishmaniasis at the POC are urgently needed. To date, there is no field-standardized molecular method based on DNA amplification coupled with Lateral Flow reading to detect leishmaniasis. Isothermal amplification by RPA (Recombinase Polymerase Amplification) is a novel strategy to diagnose infectious diseases that can be used at the POC because it is highly sensitive, fast, inexpensive and able to work at most ambient temperatures. Furthermore, RPA does not require refrigeration of reagents and can be adapted easily to lateral flow detection.

Hypothesis: *RPA coupled with a Lateral Flow test strip (RPA-LF) to detect Leishmania DNA will have high sensitivity and specificity to diagnose cutaneous leishmaniasis at the point of care in a field setting.*

Study Design. We propose to utilize for the first time an RPA-based assay coupled with lateral flow (LF) reading to diagnose cutaneous leishmaniasis. We will test novel approaches that could enhance the success of the RPA method in the field, including 1) isolation of DNA from clinical samples using a mini (portable) extractor at the POC or FTA Whatman filter paper specially designed to improve DNA preservation and purification at POC. **Aim 1: To optimize the analytical sensitivity and specificity of the genus- and complex-specific RPA-LF tests using *Leishmania* isolates and clinical samples from collaborating study sites.** We successfully developed *Leishmania spp.* primer sets for RPA that specifically amplified *Leishmania* kinetoplast DNA and were able to detect the equivalent of <10 parasites in spiked clinical specimens. We will compare the analytical sensitivity and specificity of RPA-LF with qPCR using a broad panel of clinical *Leishmania* isolates from the field sites (NAMRU-6 in Peru and NAMRU-3 detachment in Ghana). **Clinical validation:** A minimum of 20 retrospective convenience samples of clinical specimens known to be parasite positive or negative by PCR sent to UTMB from the field sites will be evaluated by RPA-LF. **Aim 2: To prospectively determine the diagnostic sensitivity and specificity of the RPA-LF test for diagnosis of cutaneous leishmaniasis.** **Sub-aim 2.1. New World CL (NAMRU-6):** A prospective field trial of the diagnostic test will be conducted in Puerto Maldonado, Madre de Dios, Peru. Based on estimated RPA-LF sensitivity of 95% and specificity of 99% we will enroll 184 positive, parasite confirmed individuals and 42 parasite negative controls to have adequate statistical power. The sensitivity and specificity will be determined using microscopy of dermal samples, and qPCR as the gold standard. **Sub-aim 2.2. Old World CL (NAMRU-3):** A similar prospective field trial will be conducted through the NAMRU-3 Ghana detachment, at the Noguchi Memorial Institute for Medical Research. Considerable effort will be taken to ensure consistency at the two sites. Patients will be enrolled principally from the villages in the Ho, HoHoe, and Kpando districts of the Volta Region where CL outbreaks due to *L. major* were previously recorded.

The repeatability of the RPA-LF test will be determined in the NAMRU's field sites while the reproducibility will be determined in the central diagnostic lab at UTMB where a subset of samples (10%) of positive and negative individuals will be delivered by the investigators of NAMRU-3 and NAMRU-6.

Training: The project will provide training to field and laboratory personnel, as well as military personnel temporarily stationed in the field of endemic areas to ensure effective deployment of the POC test.

15. SUBJECT TERMS

Cutaneous leishmaniasis-diagnosis-point of care-DNA amplification-field applicable

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1. INTRODUCTION:

Leishmaniasis is caused by the protozoan *Leishmania* and is generally transmitted by the bite of sand flies of the genus *Lutzomyia* or *Phlebotomus*. The disease has significant global impact, producing 10-20 million cases of leishmaniasis worldwide. Cutaneous leishmaniasis (CL) is characterized by chronic skin ulcers that can impact the individual's functional status, lead to expensive and untimely treatment, and result in disfiguring scarring. Military training and combat operations resulted in cases of CL in soldiers (USA, UK) deployed to Central America. More recently (2003-2004), CL was reported in almost 1,200 members of the U.S. Armed Forces deployed to Iraq, Afghanistan, and Kuwait, and the infection is an ongoing concern in the OEF/OIF veteran population. To date, there is no field-standardized molecular method based on sensitive DNA amplification coupled with Lateral Flow reading to detect leishmaniasis. Isothermal amplification by RPA (Recombinase Polymerase Amplification) is a novel strategy to diagnose infectious diseases that can be used at the POC because it is highly sensitive, fast, inexpensive and able to work at most ambient temperatures.

2. KEYWORDS:

Cutaneous leishmaniasis-diagnosis-point of care-DNA amplification-field applicable-isothermal amplification-protozoan parasite

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The overarching goal of this project is to test and evaluate a novel Leishmaniasis diagnostic (the RPA-LF) against several different parasite species. Completing this study will provide an essential product for the warfighter. Our role in this project is to identify and isolate *Leishmania* samples in South America and to test the RPA-LF with retrospective (year 1) and prospective samples (year 2 & year 3).

What was accomplished under these goals?

During the second year of the project, we continued working with our collaborating investigator, Dr. Bruno Travi, to extract DNA from cutaneous leishmaniasis samples from Peruvian patients in order to perform species-specific identification by Nested Real Time PCR FRET probes-based. In addition, as part of a training workshop, we tested a sub-set of the samples with the RPA-LF assay in our laboratory in Lima, and sent a larger sub-set of samples to the Travi laboratory at UTMB for control testing in the RPA-LF diagnostic.

During the reporting period, a total of 87 leishmaniasis samples were collected at Madre de Dios, the Amazonian region with the highest prevalence of cutaneous leishmaniasis in Peru. Moreover, as mentioned above, a training workshop in RPA-LF led by Dr. Travi and Dr. Maxy De los Santos, was conducted at NAMRU-6 during May 2-6, 2016 to train NAMRU-6 and NAMRU-3 laboratory scientist personnel. Thirteen samples were tested during this Workshop, eight of them tested positive and five tested negative for Leishmaniasis, consistent with the kDNA-PCR results, which were used as gold standard.

Additional to those 13 samples, we collected additional 74 samples and extracted DNA from 22 leishmaniasis samples (52 samples are pending of processing). Since the Scope of Work (SOW) stated that 129 participants were going to be enrolled during second year 2, NAMRU-6 has completed 67.4% of the sample projected for the reporting period (38.5 % of the study overall sample size, n=226). This work resulted in partial completion of Sub-aim 2.1 (to recruit suspicious cutaneous leishmaniasis patients and evaluate the sensitivity/specificity of RPA-LF vs. gold standard kDNA-PCR at NAMRU-6; Lima and Puerto Maldonado, Madre de Dios, Peru).

Note: It was mentioned in the year 1 annual report that the Puerto Maldonado field site required minor electrical and plumbing upgrades to use equipment required for this study. During the current reporting period those upgrades were started, but some delays in purchasing of equipment presented during year 2. We anticipate that the upgrades will be completed during the first quarter of year 3.

Specific Aim	Month	% Completion
Aim 1: To use simulated field conditions to optimize and produce the established RPA lateral flow diagnostic test for POC deployment.		
Sub-Aim 1.2: To determine if a simple DNA extraction method will provide adequate sensitivity for optimal test function under field conditions. Comparison of DNA yield, sufficient for RPA-LF test using a DNA mini-extractor vs. Whatman FTA filter paper utilizing dermal tissues spiked with <i>Leishmania</i> grown in the lab	1-3	80% Lab assays completed. Clinical samples from the field still require optimization of DNA purification
Sub-Aim 1.3: To determine if subgenus- and/or species-specific primer-probe sets can achieve the same analytical sensitivity and specificity as the genus specific primer-probe set using <i>Leishmania</i> isolates and clinical specimens from the field sites.	3-12	100% The analytical sensitivity of the RPA-LF was established for <i>Leishmania</i> (<i>Viannia</i>) spp., <i>L. major</i> and <i>L. enriettii</i>
Kickoff Coordination Meeting of participating institutions	3	100% A UTMB meeting was organized with participants of all three study sites

Protocol submission for local IRB approval and HRPO approval	3	N-6 100% N-3 90% Ghana IRBs completed; pending final N-6 approval
Implementation of molecular laboratory in Madre de Dios and technology transfer of kDNA PCR procedures from Lima to Madre de Dios for on-site Leishmaniasis diagnosis in the endemic area	6-12	80% Training completed and equipment purchased. Lab set up is awaiting final construction of dedicated facilities.
Milestone Achieved: Local IRB and HRPO approved protocols	6	UTMB 100% NAMRU-6 100% NAMRU-3 80%
Milestone(s) Achieved: Coordination meeting completed Approvals of IRBs in place to initiate field studies in human populations RPA-LF test fully adapted for field application on-site molecular diagnosis of cutaneous leishmaniasis in Madre de Dios	12	See specific items described above in the table
Aim 2: To prospectively determine the diagnostic sensitivity and specificity of the RPA-LF for diagnosis of cutaneous leishmaniasis.	12 - 36	
Sub-aim 2.1. To recruit suspicious cutaneous leishmaniasis patients and evaluate the sensitivity/specificity of RPA-LF vs. standard kDNA PCR at NAMRU-6; Lima Peru. delivery of subset of positive and negative clinical samples (10%) from NAMRU-6 to UTMB for reproducibility testing	12 - 36	74 samples have been sent to UTMB during the reporting period.

Technical meeting at NAMRU-3, Ghana	14	The technical meeting did not take place
Sub-aim 2.2. To recruit suspicious cutaneous leishmaniasis patients and evaluate the sensitivity/specificity of RPA-LF vs. standard PCR at NAMRU-3, Ghana detachment, Noguchi Memorial Institute for Medical Research, Ho Volta region Delivery of subset of positive and negative clinical samples (10%) from NAMRU-3 to UTMB for reproducibility testing	12 - 36	No participants have been enrolled at Ghana during the reporting period
Technical meeting at NAMRU-6, Peru		100% 01 training Workshop in RPA-LF developed on May 02-06, 2016

What opportunities for training and professional development has the project provided?

- During the second year of the project, both, Luis Angel Hurtado and Rocio Santos, NAMRU-6 research scientists, were trained in 1. Isolation of DNA from cutaneous leishmaniasis samples of peruvian patient collected by FTA Whatman filter paper protocol, 2. Perform identification of *Leishmania* at genus level by kDNA-PCR, and 3. determination of *Leishmania* species by Nested Real Time PCR FRET probes-based.
- In addition, as mentioned before, a training Workshop in RPA-LF took place during May 2-6, 2016.
- The participants were:
 - Dr. Bruno Travi, UTMB (trainer)
 - Dr. Maxy De Los Santos, NAMRU-6
 - Blgist. Rocio Santos, NAMRU-6
 - Blgist. Luis A. Hurtado, NAMRU-6
 - Dr. Naiki Puplampu, NAMRU-3
 - Dr. Diana Carolina Gallego, CIDEIM, Colombia
 - Blgist. Jose Luis Malaga, UPCH-UTMB
- This 05-days workshop consisted of 02 days of theory lectures, and 03 days of laboratory work, were 13 samples collected in Madre de Dios were analyzed.

How were the results disseminated to communities of interest?

During the first year of the project, Dr. Bruno Travi and Dr. Christian Baldeviano presented progress on the project at the Military Health System Research Symposium (MHSRS) in Fort Lauderdale during August 17-20, 2015. No additional dissemination activities have been held during year 2, as only few samples have been tested using the RPA-LF during this reporting period.

What do you plan to do during the next reporting period to accomplish the goals?)

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period we will continue working on specific aim #2 in the SOW in NAMRU-6, Lima. The goals of specific aim #2 (year 2 & year 3) are to prospectively test and evaluate the sensitivity and specificity of the RPA-LF in an austere field setting (Puerto Maldonado, Peru for the NAMRU-6 samples). During Workshop on RPA-LF in Lima, we discussed the necessity of enhancing conditions in NAMRU-6, Puerto Maldonado through installation of equipment and adequate infrastructure in order to perform the technique at this site. This technology transfer is planned for year three.

To prospectively test the performance of the RPA-LF, we will initiate specific aim 2.1 of the SOW. We will continue isolating patient samples in Puerto Maldonado, and performing kinetoplast DNA(kDNA) PCR when implementation of this site will be completed. The pending task, which will be conducted during the next reporting period, is to compare the results of kDNA PCR to the RPA-LF. Completing this portion of the SOW during year #3 reporting period will yield an indication of assay performance vs. the highly sensitive gold-standard (kDNA PCR). Eventual completion of this portion of the SOW is critical as it indicates the performance of the RPA-LF as a durable and potentially deployable test kit for US military personnel.

Statement of Work (SOW)

Statement of Work (SOW) – October 2014-2017

Aim 1: To use simulated field conditions to optimize and produce the established RPA lateral flow diagnostic test for POC deployment.

Sub-Aim 1.2: To determine if a simple DNA extraction method will provide adequate sensitivity for optimal test function under field conditions. Comparison of DNA yield, sufficient for RPA-LF test using a DNA mini-extractor vs. Whatman FTA filter paper utilizing dermal tissues spiked with *Leishmania* grown in the lab

Sub-Aim 1.3: To determine if subgenus- and/or species-specific primer-probe sets can achieve the same analytical sensitivity and specificity as the genus specific primer-probe set using *Leishmania* isolates and clinical specimens from the field sites.

Aim 2: To prospectively determine the diagnostic sensitivity and specificity of the RPA-lateral flow test for diagnosis of cutaneous leishmaniasis.

Sub-aim 2.1. To recruit suspicious cutaneous leishmaniasis patients and evaluate the sensitivity/specificity of RAP-Lateral Flow vs. standard kDNA PCR at NAMRU-6; Lima and Puerto Maldonado, Madre de Dios, Peru. Delivery of subset of positive and negative clinical samples (10%) from NAMRU-6 to UTMB for reproducibility testing

Sub-aim 2.2. To recruit suspicious cutaneous leishmaniasis patients and evaluate the sensitivity/specificity of RAP-Lateral Flow vs. standard PCR at NAMRU-3, Ghana detachment, Noguchi Memorial Institute for Medical Research, Ho Volta region

Delivery of subset of positive and negative clinical samples (10%) from NAMRU-3 to UTMB for reproducibility testing

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Testing the RPA-LF on retrospective Leishmania samples isolated in Peru during year one confirmed the performance of the RPA-LF. Among 74 total samples that were sent to the Travi lab for analysis during this project period. One-hundred percent of the tested samples were confirmed positive for Leishmaniasis, demonstrating that the assay has high sensitivity to detect parasite from field isolates. Importantly, these results suggest that the RPA-LF could be used to detect Leishmaniasis from US military personnel operating in South America.

At the end of year 2, additional 74 samples were sent to UTMB for analysis; those samples will be tested within the next weeks. In addition, as mentioned before, out of the 13 samples tested with the RPA-LF during the training workshop, 08 tested positive and 05 tested negative for leishmaniasis. These results were consistent with the kDNA results, thus provides additional data on the test's sensibility for detecting parasites from field isolates.

What was the impact on other disciplines?

The diagnostic method- isothermal amplification of DNA has impacted the field of molecular biology in austere environments. While the reaction mechanism is used in this project to detect cutaneous leishmaniasis, a parasite that has plagued US soldiers during the war on terror, the technology can be applied to detect a plethora of other pathogens. These pathogens include, but are not limited to: Malaria, HIV, and pox viruses.

What was the impact on technology transfer?

Testing the RPA-LF on retrospective cutaneous Leishmaniasis samples provides an additional proof of concept and only makes the RPA-LF a more enabling technology. We anticipate that the third year of the study (testing prospective samples) might attract commercial partners to help us develop this product further.

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including: transfer of results to entities in government or industry; instances where the research has led to the initiation of a start-up company; or adoption of new practices.

Not applicable at this time. However, we anticipate that completing our testing of prospective samples (during year 3 of the study) holds promise to attract commercial partners.

What was the impact on society beyond science and technology?

Cutaneous Leishmaniasis infects approximately 1.2 million people worldwide each year. Infections leave patients disfigured. Rapid identification of infections in soldiers that travel to Leishmania endemic areas would reduce treatment delays and reduce the risk of unnecessary disfigurement during deployment.

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as: improving public knowledge, attitudes, skills, and abilities; changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or improving social, economic, civic, or environmental conditions

Employment of a rapid diagnostic for cutaneous leishmaniasis could lead to more effective mass-treatment campaigns in countries that struggle with the parasite (endemically). Because the RPA-LF assay lacks a requirement for a cold chain, the system is a candidate for use in austere settings where access to refrigeration systems is lacking. Rapid identification and treatment of leishmaniasis in these austere, endemic settings could reduce lost work hours and potentially increase productivity (Gross domestic product etc.) for nations where the parasite is endemic.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

- Vince Gerbasi was changed for Carmen Lucas as PI of NMRCD.2007.0018 protocol where the present project is included.

Actual or anticipated problems or delays and actions or plans to resolve them

- Administrative issues regarding purchasing of reagents and materials from China and the UK impacted directly in the execution of RPA-LF assays in NAMRU-6, Lima.
- Delay of equipment purchasing and delivery to NAMRU-6, Puerto Maldonado as part of the site's upgrades.

Plans to solve the issues:

- International purchases have been made through the Henry M. Jackson Foundation. Once the items are cleared in customs, they will be shipped to Peru through DPO.
- In the interest of time, DNA extraction has already been started in order to expedite samples processing once the reagents/materials arrive.

Changes that had a significant impact on expenditures

- Completion of minor electrical and plumbing upgrades to use equipment and reagents in NAMRU-6, Puerto Maldonado.
- Delay in purchase of reagents and equipment.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

N/A- none.

Significant changes in use or care of human subjects

N/A- none.

Significant changes in use or care of vertebrate animals

N/A- none.

Significant changes in use of biohazards and/or select agents

N/A-none.

6. PRODUCTS

- (1) Lay Press: *Nothing to Report*
- (2) Peer-Reviewed Scientific Journals: In preparation
- (3) Invited Articles: *Nothing to Report*
- (4) Abstracts: *Nothing to Report*
- (5) Books or other non-periodical, one-time publications: *Nothing to Report*
- (6) Other publications, conference papers, and presentations: *Nothing to Report*
- (7) Website(s) or other Internet site(s): *Nothing to Report*
- (8) Inventions, patent applications, and/or licenses: *Nothing to Report*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Personnel	Role	Nearest Person Month
LCDR Sarah-Blythe Ballard	PI (Peru site)	1
Contribution: Coordinated overall research effort for project in Peru. Recruited appropriate staff to work at the Puerto Maldonado research facility. Planned research project and project modifications with project lead (Dr. Bruno Travi).		

LCDR Andrew Letizia	PI (Ghana site)	1
Contribution: Functioned as the lead PI for the project in Ghana. Supervised principal contractor (Noguchi Institute).		
Carmen Lucas, PhD	Co-Investigator	1
Contribution: Served as PI of the umbrella IRB protocol under which this project falls, coordinated transport of samples from Peru to UTMB, and amended IRB protocols for use in this project.		
LT Robert V. Gerbasi	Past-PI (Peru Site)	1
Contribution: Coordinated overall research effort for project in Peru. Recruited appropriate staff to work at the Puerto Maldonado research facility. Planned research project and project modifications with project lead (Dr. Bruno Travi).		
Gerald C. Baldeviano, PhD	Co-Investigator	1
Contribution: Worked with Dr. Travi to optimize the DNA isolation protocol, coordinated transport of samples from Peru to UTMB, and amended IRB protocols for use in this project.		
Maxy B. De los Santos, PhD	Co-investigator	2
Contribution: Functioned as the lead laboratory investigator in Peru. Trained laboratory personnel in Leishmania DNA isolation and kDNA PCR amplification and detection.		
LCDR Nehkonti Adams	Past-PI (Ghana site)	1
Contribution: Functioned as the lead PI for the project in Ghana. Supervised principal contractor (Noguchi Institute). Her efforts resulted in host country approval of the project.		

Organization Name: Noguchi Memorial Institute for Medical Research (NMIMR)

Location of Organization: Ghana

Partner's contribution to the project: Collaboration (e.g., partner's staff works with project staff on the project per the attached SOW)

Personnel	Role	Nearest Person Month
Prof. Daniel Boakye	PI	3
Contribution: Provided scientific guidance and advice in the execution of the study in Ghana, acted as main point of contact between the NAMRU-3/NAMRU-6/UTMB team and the NMIMR and as well as the Ghana Health Service/Ministry of Health.		
Clara Yeboah	Research Assistant	12
Contribution: Responsible for data entry and management as well as performing all laboratory procedures at NMIMR and the field, training field staff as well as going to the field to collect specimen and ensure that study procedures are being adhered to.		

Change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period

Nothing to Report

(**note:** the Partnering PI at NAMRU-6, Robert V. Gerbasi LT USN was replaced by LCDR Sarah-Blythe Ballard; this change has been submitted and is pending full approval)

Other organizations involved as partners

Organization Name: Fundacion Oswaldo Cruz-FIOCRUZ

Location of Organization: Brazil

Partner's contribution to the project

In-kind support: Species and strains of *Leishmania* isolated from patients in endemic areas of cutaneous leishmaniasis.

Facilities: Laboratory facilities of Dr. Renato Porrozzi at FIOCRUZ to carry out *Leishmania* identification using the RPA-LF test.

Collaboration: FIOCRUZ staff (PhD student) collaborated in the evaluation of *Leishmania* strains.

Organization Name: Centro Internacional de Entrenamiento e Investigaciones Médicas-CIDEIM

Location of Organization: Colombia

Partner's contribution to the project

In-kind support: Delivery from the lab of Dr. Nancy Gore Saravia of *Leishmania* strains isolated from patients in endemic areas of cutaneous leishmaniasis.

Organization Name: Yale School of Public health

Partner's contribution to the project

In-kind support: Delivery of *Leishmania major* strains from the lab of Dr. Diane McMahon-Pratt.

Organization Name: Lancaster University

Location of Organization: UK

Partner's contribution to the project

In-kind support: Delivery of *Leishmania major* and *Leishmania enriettii* strains from the lab of Professor Paul Bates.

8. SPECIAL REPORTING REQUIREMENTS

- Collaborative Awards

This is a collaborative award working with the University of Texas Medical Branch, PI: Bruno L. Travi (PR130282).

- Quad Charts
See appendix.

9. APPENDICES

A Novel Field-Deployable Point-of-Care Diagnostic Test for Cutaneous Leishmaniasis

Leishmaniasis

PR130282P1 and Task Title Here

PI: Sarah B. Ballard

Org: The Henry M Jackson Foundation

Award Amount: \$428,600

Study/Product Aim(s)

Aim 1: To use simulated field conditions to optimize and produce the established RPA lateral flow diagnostic test for POC deployment.

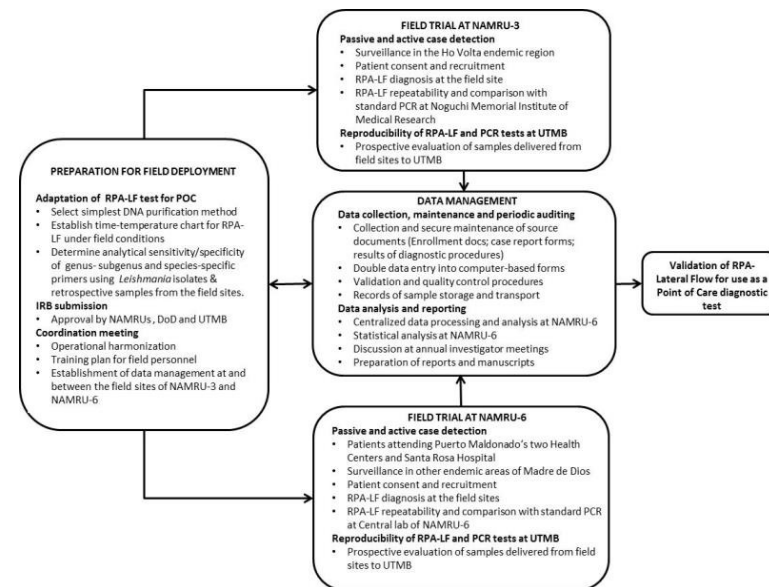
Aim 2: To prospectively determine the diagnostic sensitivity and specificity of the RPA-lateral flow test for diagnosis of cutaneous leishmaniasis.

Approach

Sub-Aim 1.1: To identify time-temperature constraints for optimal test function under field conditions. **Sub-Aim 1.2:** To determine if a simple DNA extraction method will provide adequate sensitivity for optimal test function under field conditions.

Sub-Aim 1.3: To determine if subgenus- and/or species-specific primer-probe sets can achieve the same analytical sensitivity and specificity as the genus-specific primer-probe set using *Leishmania* isolates and clinical specimens from the field. **Sub-aim 2.1.** NAMRU-6; Lima, Puerto Maldonado in Madre de Dios and Iquitos in Loreto, Peru

Sub-aim 2.2. NAMRU-3, Ghana detachment, Noguchi Memorial Institute for Medical Research, Ho Volta region.



Timeline and Cost

Activities	14	15	16	17
To identify time-temperature constraints for optimal test function under field conditions	■	■		
To determine if DNA extraction method will provide adequate sensitivity for optimal testing under field conditions	■	■		
To determine analytical sensitivity and specificity as the genus-specific primer-probe set using <i>Leishmania</i> isolates and clinical specimens from the field	■	■		
To recruit suspicious CL patients and evaluate the sensitivity/specificity of RAP-LF vs. standard kDNA PCR at NAMRU-6			■	■
Delivery of subset of positive and negative clinical samples (10%) from NAMRU-6 to UTMB for reproducibility testing			■	■
Estimated Budget (\$K)	\$0	\$0	\$0	\$0

Month 6: Local IRB and HRPO approved protocols. Coordination meeting completed- **On Track**

Month 12: Approvals of IRBs in place to initiate field studies in human populations in place. **NAMRU-6 on Track;** RPA-Lateral Flow test fully adapted for field application

On-site molecular diagnosis of cutaneous leishmaniasis in Madre de Dios, Peru set up. **Training completed. Awaiting lab setup**

YEARS 2-3: Month 14: Technical meeting at NAMRU-3-Ghana (pending)

Month 18: Training workshop held at NAMRU-6, Peru

Month 24: Overall study enrollment: 40% progress, sample to be completed during Year 3. 10% of samples to be sent to UTMB for reproducibility testing completed:

On Track

Month 36: Updated epidemiological assessment of cutaneous leishmaniasis in the endemic areas of Peru and Ghana available.

New point-of-care diagnostic test for cutaneous leishmaniasis ready for submission to obtain FDA clearance. Final Report to DoD and scientific publications of results. **(upon completion of study).**

Comment: Lab evaluations of test completed.

Updated: (Sep. 30, 2016)